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Enhanced expression of chemotactic receptors in multiple sclerosis lesions

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Abstract

We have previously shown that astrocytes and microglia express the receptors for C5a, interleukin-8 (IL-8) and *N*-formyl-Met-Leu-Phe (FMLP) in vitro. The expression and function of chemotactic receptors in the central nervous system (CNS) is, however, largely unexplored. In this study, we examined tissue sections from normal human brain and active, chronic active and chronic silent multiple sclerosis (MS) lesions for the expression of the receptors for C5a, IL-8 and FMLP by immunohistochemistry. In normal brain tissue, the expression of all three receptors was seen at low levels on astrocytes and microglia. In contrast, expression for all three receptors was markedly elevated on foamy macrophages in the acute and chronic active MS lesions. In addition, fibrous astrocytes stained intensely for the C5a receptor in the chronic active disease. Receptor expression in the chronic silent lesion was low and similar to that seen in normal brain, with staining confined to a few hypertrophic astrocytes and foamy macrophages. These are the first studies to demonstrate expression of these receptors in the CNS and elevated receptor expression in inflammatory MS lesions. The data suggest that these chemotactic receptors may play a role in inflammatory responses in MS and possibly in other CNS diseases.

Keywords: Receptor; Multiple sclerosis; Complement; Chemokine; Astrocyte; Microglia

1. Introduction

Multiple sclerosis (MS) is a debilitating condition of the human central nervous system (CNS) in which inflammatory demyelinating lesions are the hallmark feature (Raine, 1991). In recent years, it has become increasingly clear that the inflammation in MS lesions is associated with a number of immune system molecules, including a variety of proinflammatory and regulatory cytokines, adhesion molecules and complement proteins (Brosnan et al., 1995; Cannella and Raine, 1995; Shin and Koski, 1992; Morgan, 1992). From studies on laboratory models of autoimmune demyelination, it is also believed in MS, that leukocytes adhere to and transmigrate across the blood–brain barrier, infiltrating perivascular regions around lesions (Cross, 1992; Raine, 1994). The key molecules regulating these cellular trafficking events are adhesion molecules and chemotactic receptors (Brosnan et al., 1995; Carlos and Harlan, 1994; Gerard and Gerard, 1994; Murphy, 1994; Springer, 1994). While the expression of cytokines, adhesion molecules and complement in the CNS and their association with MS lesions is well documented (Brosnan et al., 1995), the expression of chemotactic receptors in the CNS has received little attention.

In all tissues, chemotaxis in inflammatory responses is mediated by a variety of substances, including C5a, a proteolytic fragment of the fifth component of complement, (Hugli, 1986), chemokines (Oppenheim et al., 1991), TGF-β (Wahl et al., 1987; Brandes et al., 1991; Morganti-Kossmann et al., 1992) and bacterial *N*-formyl peptides, such as f-Met-Leu-Phe (FMLP) (reviewed in Snyderman and Uhing, 1992). Each of these factors exerts its effect by binding to specific cell-surface receptors. Although traditionally the expression of chemotactic receptors was thought to be restricted largely to leukocytes and

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a few other cell types (reviewed in Wetsel, 1995), studies have shown that in the rat brain, microglia, the resident macrophage of the brain, and astrocytes are chemotactic for recombinant C5a (Armstrong et al., 1990; Yao et al., 1990), suggesting expression of the C5a receptor (C5aR). There is also evidence, at the mRNA level, for the expression of the IL-8R and platelet-derived growth factor receptor by glioblastoma cells (Sasahara et al., 1991; Tada et al., 1994). More recently, we have demonstrated at the protein and mRNA levels that astrocytes and microglia express receptors for C5a, IL-8, FMLP (Lacy et al., 1995). In addition, Gasque et al. (1995) have also shown that astrocytes express the C5a receptor. Collectively, these data demonstrate that the expression of many chemotactic receptors also occurs within the CNS, and it is likely that these molecules play a role during in infection and inflammatory responses in nervous tissue.

In this report, we demonstrate, for the first time, that in comparison to normal brain tissue, expression of the receptors for C5a, IL-8 and FMLP is markedly elevated in inflammatory acute and chronic active MS lesions. Elevated receptor expression appeared to be restricted primarily to foamy macrophages and reactive astrocytes. Expression of the receptors was markedly lower in the chronic silent MS lesion, and was comparable to levels seen in normal tissue. These data suggest that increased expression of the chemotactic receptors is restricted to inflammatory lesions in MS. These findings also suggest that localized, lesion-specific mechanisms regulate increased levels of receptor expression in MS lesions.

2. Materials and methods

2.1. Tissue

Fresh frozen CNS tissue was obtained within 6–8 h of death from three cases of MS, all of whom succumbed to respiratory complications. The MS patients comprised a 31-year-old female that had displayed a malignant course of chronic progressive MS of 11 years duration and whose CNS displayed evidence of recent (acute) and chronic active lesions; a 38-year-old female with typical chronic progressive MS whose CNS contained chronic active lesions; and a 51-year-old male with chronic inactive MS and a disease course of 14 years. From the CNS of these three subjects, frozen sections (6 μm) were made from blocks containing acute, chronic active and chronic silent lesions. Paraffin-embedded blocks sampled from regions adjacent to the frozen samples were sectioned and stained with H & E to confirm lesion activity. For control purposes, frozen sections of brain tissue were taken from a 62-year-old male with no history of neurologic disease who succumbed to throat cancer. This study was approved by the Institutional Review Board at the University of Alabama at Birmingham.

2.2. Lesion classification

Lesions in MS are classified as acute, chronic active or chronic silent on the basis of inflammatory activity, myelin damage and glial scarring (reviewed in Raine, 1991). Acute lesions are intensely inflammatory and edematous and are characterized by the presence of hypertrophic astrocytes and debris-laden macrophages throughout the lesion. The margin of the acute lesion is poorly defined. The chronic active lesion is an established lesion characterized by a well-defined margin along which a broad zone of perivascular and parenchymal inflammation and ongoing demyelination is superimposed. The lesion edges also contain hypertrophic astrocytes and macrophages which contain myelin debris, but the centers are intensely scarred by fibrous astrocytes which surround demyelinated axons. Chronic silent lesions are noninflammatory and readily distinguished by clearly demarcated areas of demyelination. There is invariably some axon loss in the lesion, while fibrous astrogliosis is found throughout the lesion along with the occasional foamy macrophage.

2.3. Antibodies

Rabbit anti-C5a-R, IL-8-R, FMLP-R antibodies were prepared as previously described (Haviland et al., 1995). Rabbit anti-glial fibrillary acidic protein antibody was purchased from Sigma Chemical Co. (St. Louis, MO). Murine anti-collagen type IV antibody was purchased from Jackson Immunoresearch (West Grove, PA). The murine anti-CD18 antibodies were a generous gift from Dr. Pat Bucy (Department of Pathology, University of Alabama at Birmingham). Biotinylated mouse anti-rabbit and goat anti-mouse antibodies were purchased from Southern Biotechnology (Birmingham, AL). The horseradish peroxidase-conjugated streptavidin and 6 nm gold-labeled goat anti-horseradish peroxidase antibodies were purchased from Jackson Immunoresearch (West Grove, PA).

2.4. Immunohistochemistry analysis

Samples were analyzed as described by Müller-Ladner et al. (1995) following a modified protocol established by Komminoth et al. (1992). Frozen sections were cut (6 μm), fixed for 5 min. in acetone, and incubated in 4% nonfat dry milk/2% normal rabbit/goat serum buffer for 30 min at room temperature (RT) to block nonspecific binding. Slides were incubated for 1 hr with rabbit anti-human C5aR, IL-8R or FMLPR (diluted 1:50 in 2% milk/1% horse serum buffer) followed by biotinylated mouse anti-rabbit antibody (1:600) for 30 min. at RT. The slides were incubated with horseradish peroxidase-conjugated streptavidin (1:600) for 45 min. at RT, followed by incubation with 6 nm gold-labeled goat anti-horseradish peroxidase antibodies (1:30) for 45 min. at RT. After a thorough wash in ddH_2O, the samples were fixed in 3% glutaraldehyde in cacodylate buffer (pH 7.35) for 20 min. For photochemical

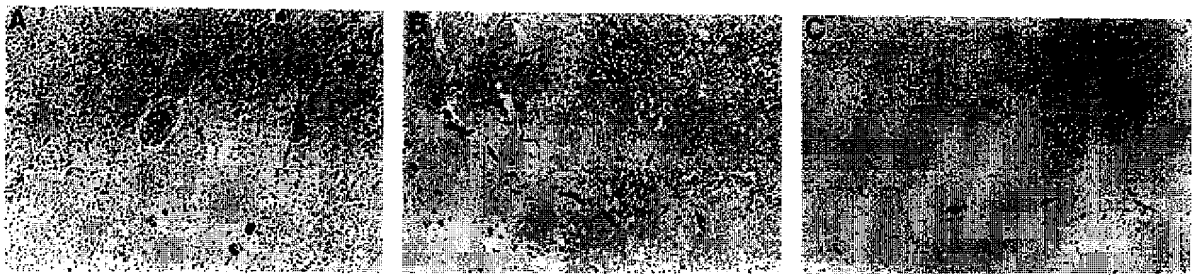

Fig. 1. H & E staining of paraffin sections of acute, chronic active and chronic silent MS lesions. (A) The plaque area of an acute lesion is shown to the left and demonstrates widespread parenchymal and perivascular infiltration by small lymphocytes. Note the many hypertrophic astrocytes within the edematous, recently demyelinated plaque area. (B) The presence of numerous small perivascular cuffs of infiltrating cells on either side of the sharply demarcated, fibrous astrogliotic chronic lesion. Plaque center is to the left and the adjacent white matter is to the right. (C) A chronic silent lesion lacking inflammatory activity is shown. The adjacent myelinated white matter can be seen above. There may be some increase in glial cells (oligodendroglial hyperplasia) evidenced by the increased number of small dark nuclei along the lesion edge.

silver amplification, slides were incubated in 0.025 M citrate buffer (pH 3.8) containing 2.5 mg/ml hydroquinone for 5 min and then in the same buffer containing 1 mg/ml silver acetate for 10–15 min. Slides were then rinsed, fixed for 2 min. (Kodafix, 1:10; Eastman Kodak, Rochester, NY) and mounted with Gelmount (Biomedia, Foster City, CA). Photographs were taken with a Zeiss microscope using Kodak film. Staining using an non-immune rabbit serum as the primary antibody was used as a control. All slides were read by two blinded observers.

3. Results

3.1. H & E staining of acute, chronic active, and chronic silent MS lesions

Fig. 1 shows representative results of H & E staining of the different MS lesion types. The margin of an acute inflammatory MS lesion is shown in Fig. 1A. The edge of a chronic active lesion is shown in Fig. 1B, while in Fig.

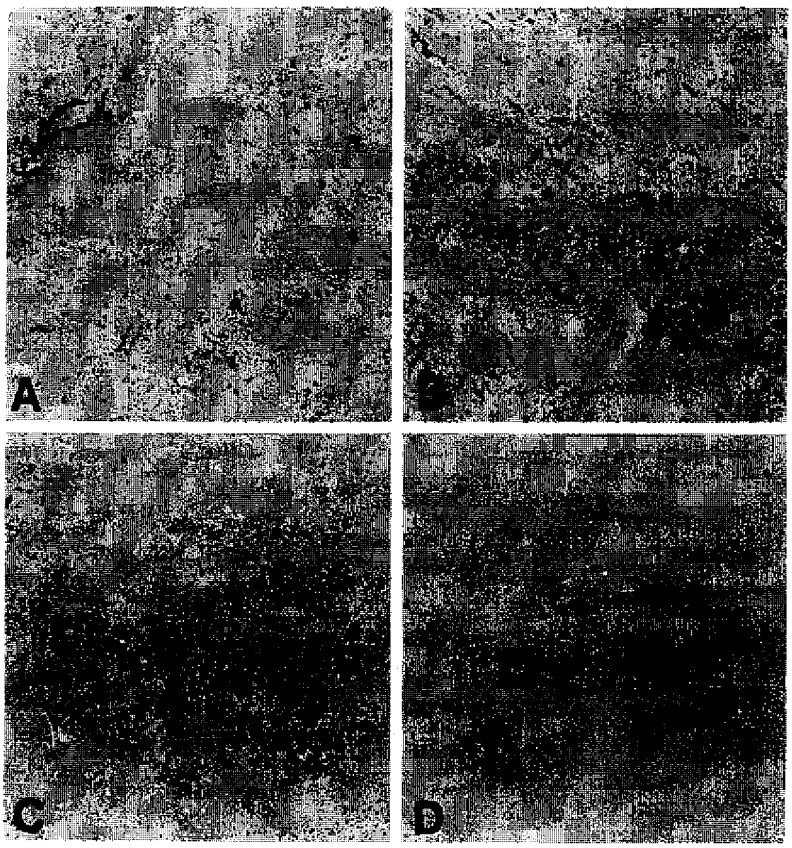

Fig. 2. Immunohistochemical characterization of C5aR, IL-8R and FMLPR expression in normal brain. Frozen sections were stained with antibody to the C5aR (A), IL-8R (B), FMLPR (C) or non-immune rabbit antiserum (D) as described in the materials and methods using an immunogold-silver staining procedure. All views are 50×.

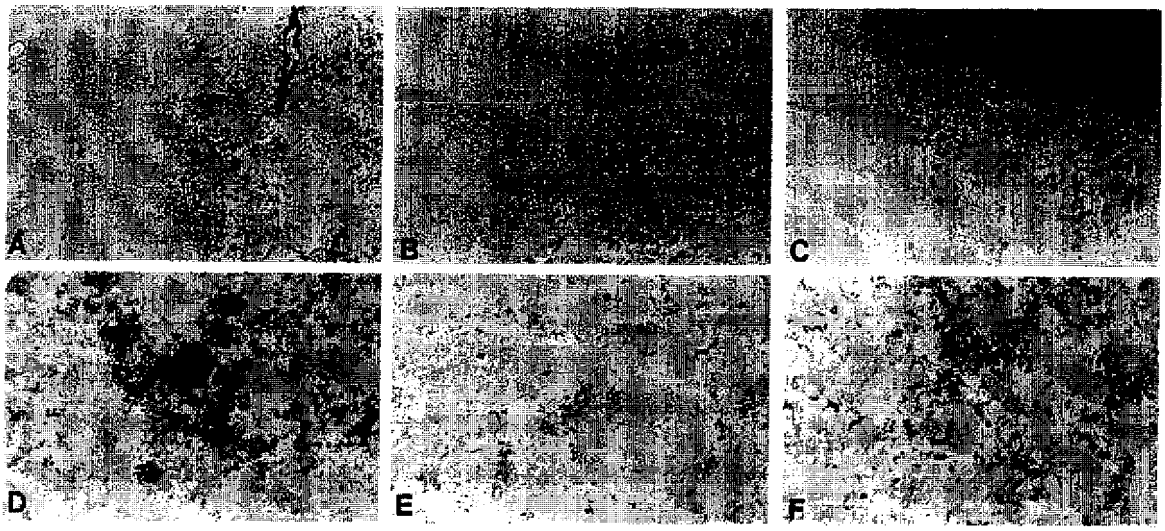

Fig. 3. The edge of an acute multiple sclerosis lesion stained for the C5aR (A and D), IL-8R (B and E) or FMLPR (C and F). Panels A–C are 50×, panels D–F are 300×.

1C, the perimeter of a chronic silent, non-inflammatory, demyelinated fibrous lesion is shown.

3.2. Expression of C5a, IL-8 and FMLP receptors in normal brain tissue

Immunohistochemical analysis of the expression of the receptors for C5a, IL-8 and FMLP in normal brain, revealed a low level of staining for all three receptors throughout all sections examined (Fig. 2A–C). Staining was confined to a few astrocytes and microglia. There was weak staining for the FMLPR in some of the blood vessels infiltrating the tissue (Fig. 2C), otherwise the endothelium was largely negative for the expression of the receptors. The application of non-immune rabbit serum instead of anti-receptor antibody, as a control, showed a very low level of background staining and demonstrated that the rabbit antibodies are specific (Fig. 2D). As would be expected, the sections were intensely positive for GFAP and showed scattered staining for CD18 (the β-chain of CR3 and CR4, common microglia markers), throughout the tissue (data not shown).

3.3. Expression of C5a IL-8 and FMLP receptors in acute and chronic active MS lesions

In contrast to the low level of staining for the receptors seen in normal brain tissue, intense staining for the receptors was observed in acute lesions (Fig. 3A–C). The staining pattern demarcated the edge of the lesion for all three receptors and was elevated throughout the lesion but not the surrounding tissue. Strong staining for the receptors was also seen along blood vessels, suggesting that endothelial expression was also elevated in the acute lesion (Fig. 3A–C). Based on morphology, expression of the receptors appears strongest on foamy macrophages scattered throughout the lesion (Fig. 3D–F).

More intense staining for all three receptors was observed in chronic active lesions (Fig. 4A–C). As in the acute lesion, the staining was localized primarily to foamy macrophages for all three receptors; however there was

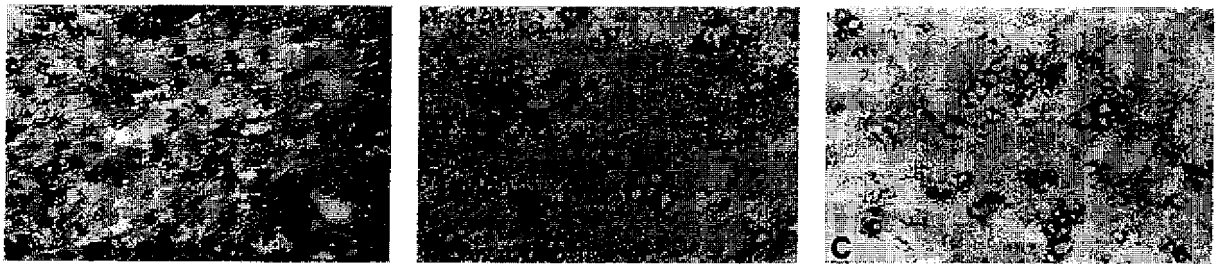

Fig. 4. Immunohistochemical staining of a chronic active multiple sclerosis lesion for the C5aR (A), IL-8R (B) or FMLPR (C). All views are 300×.

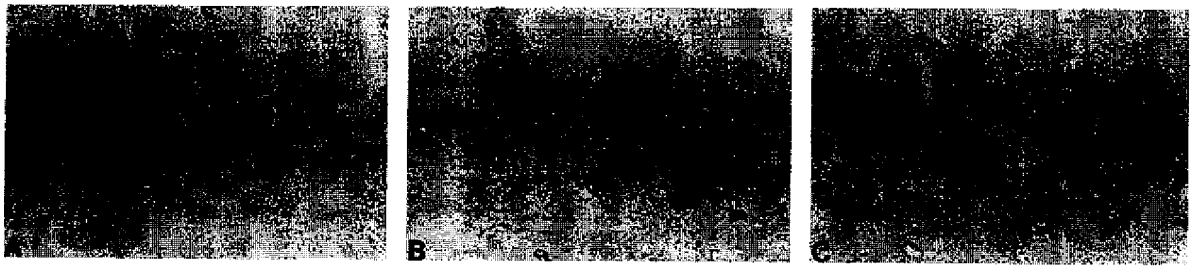

Fig. 5. Immunohistochemical staining of a chronic silent multiple sclerosis lesion for the C5aR (A), IL-8R (B) or FMLPR (C). All views are 125×.

also significant staining for the C5aR on fibrous astrocytes (Fig. 4A).

3.4. Expression of C5a, IL-8 and FMLP receptors in chronic silent MS lesions

Examination of chronic silent lesions revealed markedly lower receptor expression compared to acute and chronic active lesions (Fig. 5A–C). Receptor expression in the chronic silent lesion appeared comparable to that seen in normal tissue (compare to Fig. 2) and, the lesion proper or lesion edge could not be identified by the pattern of receptor staining. There was weak to moderate staining for the receptors on some blood vessels suggesting residual receptor expression on the endothelium (Fig. 5A–C).

4. Discussion

In this study, we have shown that the receptors for C5a, IL-8 and FMLP are expressed at low levels in normal brain tissue, similar to what we and others have previously reported for human astrocytes and microglia, in vitro (Lacy et al., 1995; Gasque et al., 1995). In contrast, receptor expression is significantly increased in acute and chronic active MS lesions. These inflammatory lesions are generally characterized by reactive astrocytes, the presence of infiltrating mononuclear cells, demyelination of axons, increased cytokine and adhesion molecule expression and deposition of complement activation components (Shin and Koski, 1992; Morgan, 1992; Brosnan et al., 1995; Cannella and Raine, 1995). In the chronic silent MS lesion, the expression of the receptors was close that seen in normal tissue, suggesting that elevated receptor expression correlates with the inflammatory stages of MS. Chemotactic receptor expression was not elevated in Alzheimer's brain tissue (S.R. Barnum, J. Jones and J. Rogers, unpublished observations). This latter finding suggests disease specificity with respect to changes in expression of the chemotactic receptors and that receptor expression may not be elevated in all inflammatory conditions in the CNS.

The mechanisms regulating the expression of the receptors in the inflammatory, demyelinating lesions are unclear at present. Information on the regulation of expression of the C5a and FMLP receptors on most cell types is very limited. Expression of the C5aR and FMLPR on U937 cells, a human monocytic cell line, or HL-60 cells, a human promyelocytic cell line, is increased when the cells are developmentally induced using dibutryl-cAMP, vitamin D metabolites, cAMP agonists, phorbol esters and other agents (Harris and Ralph, 1985; Barker et al., 1986; Collins, 1987; Rubin et al., 1986; Rubin et al., 1988; Rubin et al., 1991). Regulated expression of the C5a and FMLP receptors has also not been well investigated with the exception of a report demonstrating that IFN-γ induces the expression of the C5aR in U937, HL-60 and Mono-Mac6 cells (Burg et al., 1995). Recent studies indicate that expression of the C5aR on human astrocytes is not altered by PMA, IFN-γ, IL-1β or TNF-α (Gasque et al., 1995). We have also shown that PMA and dibutryl-cAMP do not affect the expression of the C5aR, as well as, the IL-8 and FMLP receptors on human astrocytes and various astroglioma cell lines at the mRNA level (J. Jones and S.R. Barnum, unpublished observations). However, preliminary studies from our laboratory demonstrate that recombinant C5a, IL-1β or IL-8 induce C5aR expression, in a dose-dependent fashion on CRT cells, an astroglioma cell line (Ransohoff et al., 1991), as determined by flow cytometry (S.R. Barnum, J. Jones and R.A. Wetsel, unpublished observations). This data suggests that activation of complement (leading to C5a generation) or the production of proinflammatory cytokines, may participate in regulation of chemotactic receptor expression on astrocytes, and possibly on microglia or infiltrating mononuclear cells. If so, these mediators may account for at least part of the mechanism involved in elevation of chemotactic receptor expression in inflammatory MS lesions. We are extending these studies to determine if IL-8R and FMLPR expression on astrocytes is regulated in a similar fashion and in animal model systems as well.

Understanding the regulation of expression of chemotactic receptors by astrocytes, microglia and other CNS cell types is important because of the multi-functional role the receptors may mediate in CNS inflammation. The expression of C5a, IL-8 and FMLP receptors in the CNS would allow endogenously produced complement (C5a), cytokines, (IL-8) and cellular debris (mitochondrially derived FMLP; Carp, 1982) to contribute directly to inflammation through effector functions mediated by these recep-

tors. These functions include induction of cytokine and acute phase protein production, increased adhesion molecule expression, and promotion of the respiratory burst (Hugli, 1986; Okusawa et al., 1987; Okusawa et al., 1988; Montz et al., 1990; Morgan et al., 1992; Ember et al., 1994; Foreman et al., 1994; McCoy et al., 1995; Wetsel, 1995). Further, activation of endothelial cells expressing these receptors (Foreman et al., 1994), may potentially alter the integrity of the blood–brain barrier and facilitate the recruitment of potentially damaging lymphoid cells into the CNS. Should astrocytes and microglia mediate some or all of the immune-effector functions regulated by chemotactic receptors on neutrophils, monocytes and other cell types, chemotactic receptor expression in the CNS may readily contribute to initiating and augmenting inflammatory responses. This paradigm suggests that inhibiting receptor function may be a promising therapeutic target for MS and possibly other inflammatory conditions in the CNS such as trauma or infection.

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